

NO:7), Asn-Lys-Ile-Ser-Tyr-Gln (SEQ ID NO:8), and Ala-Thr-Lys-Ser-Lys-Gln (SEQ ID NO:9). Some examples of preferred heptaamino acid sequences are Glu-His-Ser-Ser-Lys-Leu-Gln (SEQ ID NO:10), Gln-Asn-Lys-Ile-Ser-Tyr-Gln (SEQ ID NO:11), and Glu-Asn-Lys-Ile-Ser-Tyr-Gln (SEQ ID NO:12). As noted, further amino acids can comprise X<sub>1</sub>--

*B1*  
*cmf*  
Replace the paragraph beginning at page 23, line 14, with the following rewritten

paragraph:

*B2*  
--Each assay contains 200 picomoles of the particular protease and 0.2 mM concentration of the particular substrate (*i.e.*, 40,000 picomoles/200 µL of assay volume). The amino acid sequences expressed in one letter amino acid code represent the following amino acid sequences in the three letter amino acid code: EHSSKLQ (Glu-His-Ser-Ser-Lys-Leu-Gln; SEQ ID NO:10), QNKISYQ (Gln-Asn-Lys-Ile-Ser-Tyr-Gln; SEQ ID NO:11), ENKISYQ (Glu-Asn-Lys-Ile-Ser-Tyr-Gln; SEQ ID NO:12), and ATKSKQH (Ala-Thr-Lys-Ser-Lys-Gln-His; SEQ ID NO:13). Determinations listed in the table were carried out with substrates at 0.2 mM concentration in the PSA assay buffer (50mM Tris/0.1M NaCl pH 7.8). The entry "UD" stands for "undetectable" and means that at most, 0.1 pmole of substrate cleavage per minute per 200 pmole protease took place. Asterisks represent experiments not performed. Abbreviations are as follows: PSA (prostate specific antigen), Chymo (chymotrypsin), Urokin (urokinase), TPA (tissue plasminogen activator), Thromb (thrombin), Kallik (human kallikrein, hK1).--

*B3*  
Replace the paragraph beginning at page 24, line 11, with the following rewritten  
paragraph:

--The amino acid sequences expressed in one letter amino acid code represent the following amino acid sequences in the three letter amino acid code: EHSSKLQ (Glu-His-Ser-Ser-Lys-Leu-Gln; SEQ ID NO:10), HSSKLQ (His-Ser-Ser-Lys-Leu-Gln; SEQ ID NO:7), SKLQ (Ser-Lys-Leu-Gln; SEQ ID NO:1), and ATKSKQH (Ala-Thr-Lys-Ser-Lys-Gln-His; SEQ ID NO:13). Determinations listed in the table were carried out with substrates at 0.2 mM concentration in the PSA assay buffer (50mM Tris/0.1M NaCl pH 7.8).--

Replace the paragraph beginning at page 25, line 10, with the following rewritten paragraph:

B4  
--The amino acid sequences expressed in one letter amino acid code represent the following amino acid sequences in the three letter amino acid code: EHSSKLQ (Glu-His-Ser-Ser-Lys-Leu-Gln; SEQ ID NO:10), QNKISYQ (Gln-Asn-Lys-Ile-Ser-Tyr-Gln; SEQ ID NO:11), ENKISYQ (Glu-Asn-Lys-Ile-Ser-Tyr-Gln; SEQ ID NO:12), and ATKSKQH (Ala-Thr-Lys-Ser-Lys-Gln-His; SEQ ID NO:13). Determinations listed in the table were carried out with substrates at 0.2 mM concentration in the PSA assay buffer (50mM Tris/0.1M NaCl pH 7.8). AMC is 7-amino-4-methylcoumarin. The human serum used was 100% for each assay. The entry "UD" stands for "undetectable", and means that not more than 0.01 picomole of substrate per minute was cleaved.--

Replace the paragraph beginning at page 25, line 20, with the following rewritten paragraph:

B5  
--A family of peptide substrates based upon the EHSSKLQ (SEQ ID NO:10) sequence was assayed for activity for the intracellular proteases, and the results given in Table 4.--

Replace the paragraph beginning at page 25, line 32, with the following rewritten paragraph:

B6  
--The amino acid sequences expressed in one letter amino acid code represent the following amino acid sequences in the three letter amino acid code: EHSSKLQ (Glu-His-Ser-Ser-Lys-Leu-Gln; SEQ ID NO:10), HSSKLQ (His-Ser-Ser-Lys-Leu-Gln; SEQ ID NO:7), and SKLQ (Ser-Lys-Leu-Gln; SEQ ID NO:1). The shorter sequences are formed by deleting amino acids from the amino terminal side of the sequence. Determinations listed in the table were carried out with substrates at 0.2 mM concentration in the PSA assay buffer (50mM Tris/0.1M NaCl pH 7.8), except SKLQ (SEQ ID NO:1), KLQ and LQ, which were carried out in 1.4% acetonitrile/buffer and Q-AMC which was carried out in 0.2% formic acid/buffer, at pH 7.8. The entry "UD" stands for "undetectable" and means that at most, 0.1 pmole of substrate cleavage per minute per 200 pmole protease took place. Asterisks represent experiments not performed.

Applicant : John T. Isaacs et al.  
Serial No. : 09/588,921  
Filed : June 7, 2000  
Page : 4

Attorney's Docket No.: 07265-149003

B6 cont Abbreviations are as follows: PSA (prostate specific antigen), Cath B, C, D (Cathepsins, B, C, D), Esterase (porcine liver esterase).--

Replace the paragraph beginning at page 40, line 12, with the following rewritten paragraph:

B7 --The Mu-HSSKLQ-AMC (SEQ ID NO:7) substrate was custom synthesized by Enzyme Systems Products (Dublin, CA) and characterized as described in Denmeade *et al.*, *Cancer Res.*, 57:4920-4926, (1997). Doxorubicin (Dox) prodrugs [Ac-His-Ser-Ser-Lys-Leu-Gln-Dox (HSSKLQ-Dox; SEQ ID NO:7) where Ac is acetyl] and [His-Ser-Ser-Lys-Leu-Gln-Leu-Dox (Mu-HSSKLQ-Leu-Dox; SEQ ID NO:14) where Mu is morpholinocarbonyl] were synthesized by coupling the primary amine of doxorubicin to the carboxyl group of the C-terminal amino acid. Purification of both compounds by HPLC yielded the trifluoroacetate salt (>98% purity). The peptide sequence was confirmed by amino acid analysis and molecular weights were confirmed by mass spectroscopy.--

Replace the paragraph beginning at page 40, line 27, with the following rewritten paragraph:

B8 --Table 6 shows the clonogenic survival of TSU-Pr1 cells following 48 hours of treatment with Mu-His-Ser-Ser-Lys-Leu-Gln-Leu-doxorubicin (SEQ ID NO:14) prodrug with and without 30 µg/ml enzymatically active PSA. Results for Mu-His-Ser-Ser-Lys-Leu-Gln-doxorubicin (SEQ ID NO:7) at 50 µM, were 122 colonies for treatment without PSA, and 110 colonies for treatment with 30 µg/ml enzymatically active PSA. Results are shown as averages (n = 5) with standard error of 2 to 7. Assays were done in triplicate.--

In the claims:

Amend claims 30 and 31 as follows:

B9 18. (Amended) The composition of claim 19, wherein the peptide is His-Ser-Ser-Lys-Leu-Gln-Leu (SEQ ID NO:14).